

hand. Electroosmotic pumps are also provided. Such pumps can be used in place of external drives to propulse the flow of solubilized reagents and sample in microfluidic device-based assays.

[0054] Blister pack: an on-board reagent pack or sachet under a deformable (or elastic) diaphragm. Used to deliver reagents by pressurizing the diaphragm and may appose a “sharp”, such as a metal chevron, so that pressure on the diaphragm ruptures the “pillow” (see pillow). These may be used with check valves or closable vents to produce directional fluid flow and reagent delivery. Elastic diaphragms are readily obtained from polyurethane, polysilicone and polybutadiene, and nitrile for example (see elastomer). Deformable, inelastic diaphragms are made with polyethylene terephthalate (PET), mylar, polypropylene, polycarbonate, or nylon, for example. Other suitable materials for the deformable film include parafilm, latex, foil, and polyethylene terephthalate. Key factors in selecting a deformable film include the yield point and the deformation relaxation coefficient (elastic modulus).

[0055] Use of these devices permits delivery or acceptance of a fluid while isolating the contents of the microfluidic device from the external environment, and protecting the user from exposure to the fluid contents.

[0056] Single entry: refers to a microfluidic device, card or cartridge that requires, or permits, only a single introduction of sample, and the assay is then conducted within a self-contained, sealed system. Such devices optionally contain a device for sealing or locking the sample port and an on-board means for disinfecting the contents of the device and any waste following completion of the assay. In one embodiment, the device can be discarded after use without special precautions.

[0057] Waste chamber or “pack”: is a cavity or chamber that serves as a receptacle for sequestering discharged sample, rinse solution, and waste reagents. Typically also includes a wicking material (see wick). Waste packs may also be sealed under an elastic isolation membrane sealingly attached to the body of the microfluidic device. This inner membrane expands as the bibulous material expands, thus enclosing the waste material. The cavity outside the isolation membrane is vented to atmosphere so that the waste material is contained and isolated. Waste packs may optionally contain dried or liquid sterilants.

[0058] Vent: a pore intercommunicating between an internal cavity and the atmosphere. A “sanitary” or “isolation vent” also contains a filter element that is permeable to gas, but is hydrophobic and resists wetting. Optionally these filter elements have pore diameters of 0.45 microns or less. These filters function both in forward and reverse isolation. Filter elements of this type and construction may also be placed internally, for example to isolate a valve or bellows pump from the pneumatic manifold controlling it.

[0059] Herein, where a “means for a function” is described, it should be understood that the scope of the invention is not limited to the mode or modes illustrated in the drawings alone, but also encompasses all means for performing the function that are described in this specification, and all other means commonly known in the art at the time of filing. A “prior art means” encompasses all means for performing the function as are known to one skilled in the art at the time of filing, including the cumulative knowledge in the art cited herein by reference to a few examples.

[0060] Means for extracting: refers to various cited elements of a device, such as a solid substrate, filter, filter plug, bead bed, frit, or column, for capturing target nucleic acids from a biological sample, and includes the cumulative knowledge in the art cited herein. Extracting further comprises methods of solubilizing, and relates to the resuspension of cells and tissue from the tip of a swab. This includes methods, for example, for dissolution of mucous and protein as described in United States Patent Application 2004/0175695 to Debad. Generally, extraction means include a mechanical pumping component that promotes physical resuspension by turbulent or near turbulent flow. Such flow may be reciprocating flow, and may be pulsatile at varying frequencies to achieve the desired resuspension in a reasonable interval of time. Extraction means also include use of detergent-based buffers, sulfhydryl-reducing agents, proteolytics, chaotropes, passivators, and other solubilizing means.

[0061] A means for polymerizing, for example, may refer to various species of molecular machinery described as polymerases and their cofactors and substrates, for example reverse transcriptases and TAQ polymerase, and includes the cumulative knowledge of enzymology cited herein by reference to a few examples.

[0062] Means for Amplifying: The grandfather of this art is the “polymerase chain reaction” (referred to as PCR) which is described in detail in U.S. Pat. Nos. 4,683,195, 4,683,202 and 4,800,159, Ausubel et al. *Current Protocols in Molecular Biology*, John Wiley and Sons, Baltimore, Md. (1989), and in Innis et al., (“PCR Protocols”, Academic Press, Inc., San Diego Calif., 1990). Polymerase chain reaction methodologies require thermocycling and are well known in the art. Briefly, in PCR, two primer sequences are prepared that are complementary to regions on opposite complementary strands of a target sequence. An excess of deoxynucleoside triphosphates are added to a reaction mixture along with a DNA polymerase, e.g., Taq polymerase. If the target sequence is present in a sample, the primers will bind to the target and the polymerase will cause the primers to be extended along the marker sequence by adding on nucleotides. By raising and lowering the temperature of the reaction mixture, the extended primers will dissociate from the template to form reaction products, excess primers will bind to the template and to the reaction products and the process is repeated. By adding fluorescent intercalating agents, PCR products can be detected in real time.

[0063] Other amplification protocols include LAMP (loop-mediated isothermal amplification of DNA) reverse transcription polymerase chain reaction (RT-PCR), ligase chain reaction (“LCR”), transcription-based amplification systems (TAS), including nucleic acid sequence based amplification (NASBA), “Rolling Circle”, “RACE” and “one-sided PCR”.

[0064] These various non-PCR amplification protocols have various advantages in diagnostic assays, but PCR remains the workhorse in the molecular biology laboratory and in clinical diagnostics. Embodiments disclosed here for microfluidic PCR should be considered representative and exemplary of a general class of microfluidic devices capable of executing one or various amplification protocols.

[0065] Means for detecting: as used herein, refers to an apparatus for displaying an endpoint, i.e., the result of an assay, and may include a detection channel and test pads, and a means for evaluation of a detection endpoint. Detection endpoints are evaluated by an observer visually in a test field, or by a machine equipped with a spectrophotometer, fluorom-